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KINETICS OF DEËSTERIFICATION OF PECTIN[1]

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The kinetics of deësterification of pectin has assumed practical and scientific importance with the growth of interest in pectins of low degree of esterification. Data on rates make possible the selection of industrial operating conditions for lowest cost and highest quality of product. Activation energies derivable from rate data also throw light on unanswered questions concerning the structure of pectin.

Pectin is a complex polysaccharidic material and in any chemical process may undergo several reactions simultaneously. Demethylation of pectin may be accompanied by chain degradation, removal of ballast,[3] oxidation, and opening of pyranose or furanose rings. In this paper, kinetics of demethylation by acid catalysis and by tomato pectase is described, and a method for determining the rate of ballast removal is discussed. In a subsequent paper, degradation of pectin will be considered fully. Kinetics of demethylation by alkaline catalysis and by citrus pectase has already been described by others (21, 22, 29).

The extent of the deësterification reaction can, in general, be followed by measuring either the loss in methyl ester or the gain in free carboxyl groups. In acid deësterification, direct titration with alkali of the liberated carboxyls in the reaction mixture is not feasible, because the change in the number of carboxyls is only an extremely small fraction of the total titratable acidity of the mixture. Direct Zeisel determination of the methyl ester content of the reaction mixture is also not possible because of interference from the liberated methanol. Hence the pectin must be precipitated from the reaction mixture and washed free of acid and methanol before analytical determinations can be made.

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[3] For the purposes of this paper, the word *ballast* will be defined as any non-uronide organic material in a sample of pectin whether or not attached to the polygalacturonide chain, in general agreement with Olsen (28) and Schneider (31).

Since some of the ballast material is lost during the washing process, the carboxyl and methyl ester contents of the precipitated and washed samples bear no simple relation to the concentrations of carboxyl and methyl ester in the reaction mixture. However, it can be shown that the degree of esterification (14) is proportional to the concentration of methyl ester in the reaction mixture and is independent of the quantity of ballast or other inert substance present. For these reasons, the degree of esterification was used in this work for following the extent of the demethylation reaction.

The amount of ballast associated with a given weight of polygalacturonide in a sample purified by washing is always less than that associated with the same weight of polygalacturonide before removal from the reaction mixture. However, if the removal of ballast follows a first-order law, and if each sample is purified under such conditions that the same *fraction* of the contained ballast is washed out, then it can be shown that the rate constant calculated from the ballast content of the purified samples will be equal to the rate constant that would have been obtained if the ballast content of the reaction mixtures could have been determined directly. Hence in this investigation the washing procedure was standardized, and the ballast content of the purified samples was used for following the extent of the ballast-removal reaction. By similar reasoning it can be seen that ballast lost in the purification of the original raw material will not affect the rate constants, provided the same batch of purified raw material is used for all the experiments of a given series. Because of small uncontrollable variations in the amount of ballast removed by washing, the data on ballast removal will of course have a higher probable error than that for the demethylation reaction.

EXPERIMENTAL

Preparation of starting materials

For one series of deësterifications (H84, H85, H86, H87, H88, H89, H91) pectin was extracted from a good grade of commercial apple pomace and purified by several precipitations from water solution with alcohol, in order to remove electrolytes and organic material of low molecular weight. The final precipitates were washed several times with 70 per cent alcohol, and about 80 per cent of the liquid was pressed out. The moist precipitates were used as starting materials for the deësterifications. For another series (H103, H106) commercial 200-grade apple pectin[4] was used without further purification. The pectins prepared by these methods and used as starting materials for the various deësterifications were all approximately 200-grade and analyzed in the following ranges: polygalacturonide content, 66–78 per cent; methoxyl, 7.5–9.4 per cent; degree of esterification, 0.63–0.80; ash content, 0.25–1.5 per cent; ash alkalinity, 0.027–0.44 milliequivalent per gram of pectin.

Acid deësterification

Acid-catalyzed deësterifications were carried out at temperatures from 30° to 60°C., at hydrochloric acid concentrations from 0.1 to 1.0 *N*, and at pectin con-

[4] Kindly furnished by Speas Company, Kansas City, Missouri.

centrations from 0.5 to 2.5 per cent. Pectin purified as above was dissolved in water and diluted to the desired concentration. The solution was placed in a constant-temperature bath (regulated to ±0.1°C.) and allowed to come to the reaction temperature before final adjustment of volume. An amount of concentrated hydrochloric acid calculated to give the desired normality was added to the solution, and time was counted from this moment. The solution was vigorously stirred at all times. Portions of the reaction mixture were removed at intervals, immediately precipitated with twice the volume of 80 per cent alcohol, and strongly stirred to break up the lumps of gel that were formed. The precipitate was filtered and washed with 80 per cent alcohol until the filtrate showed no turbidity with silver nitrate. A uniform washing procedure was used in all cases, so that the amounts of easily removable ballast washed out would be comparable. The samples were then washed with absolute alcohol, pressed, dried for 1 day at room temperature, and then dried for 1 or 2 days at 60°C. in a mechanical convection oven. Finally, the dried samples were ground to pass a 40-mesh screen and analyzed for ash content, alkalinity of the ash, carboxyl content, and methyl ester content.

Enzyme deësterification

Enzyme-catalyzed deësterifications were carried out at temperatures from 30° to 50°C. and at a pectin concentration of 1.2 per cent. The procedure was similar to that described above, except that tomato pectase was used as catalyst instead of hydrochloric acid and that a pH of 6.00 ± 0.05 was maintained by continuous addition of 2 *N* sodium hydroxide with rapid stirring. Because of the speed of the enzyme-catalyzed reaction, a fresh reaction mixture was made up for each sample, and the reaction was stopped at the desired time by quickly lowering the pH of the whole solution to 3.0 with hydrochloric acid, at which pH the enzyme is inactive. Finally, the enzyme was destroyed by heating the solution to 65°C. for 20 min., after which the product was purified in the same manner as the acid-deësterified samples.

Preparation of the enzyme catalyst

Firm, ripe tomatoes were ground to a pulp, the pH adjusted to 6.0 (37), and the juice expressed from the pulp. Suspended material and pigment were removed by decantation and filtration. The clear, yellow solution containing the pectase was stored at 0°C. under a layer of xylene as a preservative. In the deësterifications, 44 ml. of this preparation was used for each liter of 1.2 per cent pectin solution.

Figures 1 and 2 show the variation of the activity of this enzyme preparation with pH and temperature, as determined by a method to be described in another paper (12). The relation of the enzyme activity, at the pH used for deësterification, to the maximum activity of the enzyme can be seen from figure 1. From figure 2 it can be seen that all the deësterifications were run at temperatures below that at which significant denaturation of the enzyme occurs.

Analytical methods

The methyl ester content was determined by a Zeisel procedure, modified by Clark (4), upon samples treated with water vapor at low pressure to remove adsorbed alcohol (13, 17). The carboxyl content was determined by titration with sodium hydroxide to pH 7.5 (35). Ash content was determined by ignition

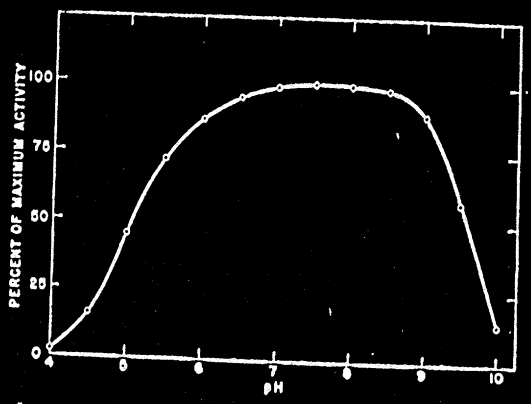

FIG. 1. Activity of tomato pectase as a function of pH. Temperature, 30°C.; total ionic strength, 0.06.

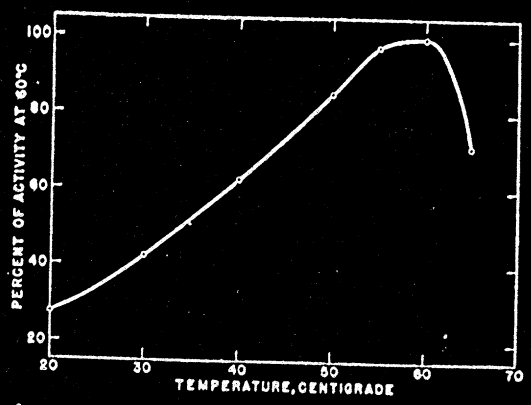

FIG. 2. Activity of tomato pectase as a function of temperature. pH, 6.5; time of reaction, 10 min.; total ionic strength, 0.06.

to constant weight at 600°C. The weights of all samples were corrected for the percentage of ash, and all carboxyl contents were corrected for the alkalinity of the ash.

Calculations

The degree of esterification (14) was calculated from its defining equation:

$$\lambda = \frac{COOCH_3}{COOH_3 + COOH} \qquad (1)$$

where $COOCH_3$ and $COOH$ are the methyl ester and carboxyl contents, respectively, in moles per gram of the solid sample.[5]

The weight per cent of polygalacturonide was calculated by the expression

$$\%P = 100(176.12 \times COOH + 190.15 \times COOCH_3) \quad (2)$$

and the weight per cent of ballast material by the expression

$$\%B = 100 - \%P \quad (3)$$

as described by Hills and Speiser (14).

In acid deësterification, the hydrogen-ion and water concentrations were sufficiently high to be unaffected by the production of carboxyl groups during the reaction. Also, because of the low concentration of the pectin, the concentration of the hydrolysis product, methanol, was always low enough for the reverse reaction to be neglected. Therefore, changes in the rate of demethylation were due solely to changes in the methyl ester concentration, and a pseudo first-order law could be assumed to describe the rate. To insure that these assumptions were applicable, rate calculations were confined to the early part of the reaction, where log λ was a linear function of time. For acid deësterification this linearity extended down to a degree of esterification of approximately 50 per cent.

The rate constant, k, was determined from the slope of log λ *versus* time by the method of weighted least squares, using the statistical methods of Birge (1). Several Zeisel values were determined for each sample, and an individual value of log λ was calculated for each Zeisel value. In this calculation, a single average value of carboxyl content was used for each sample, because the determination of carboxyl is considerably more precise than the determination of methyl ester. The several values of log λ for each sample were averaged, and the average was weighted according to the spread of the individual values, using the formulae of Birge. The probable error of k was also calculated by the method of Birge. The Arrhenius activation energy, μ, and its probable error were calculated from the slope of log k *versus* $1/T$ by the least squares procedure described above. The data for ballast removal were handled similarly.

In enzyme deësterification, calculations were made according to both the zero-order and first-order equations. Linearity of both λ and log λ *versus* time extended down to a degree of esterification of approximately 30 per cent. Otherwise, the calculation of enzyme data was performed in the same manner as for acid deësterification.

RESULTS

Figures 3 and 4 show the variation of degree of esterification and ballast content with time for a typical acid-catalyzed demethylation and a typical enzyme-catalyzed demethylation. The same starting material was used in both cases. It can be seen that in acid deësterification, ballast is lost at a rate comparable to

[5] In the acid behavior of pectin, $\Gamma = 1 - \lambda$ is the significant quantity (35).

that of demethylation, whereas in enzyme deësterification the rate of ballast removal is much less than that of demethylation. Figure 3 also shows that the ballast content is still decreasing at an appreciable rate after 144 hr., although it has fallen to 6.14 per cent. In other experiments continued for longer periods of time (14), ballast contents have been obtained as low as 0.9 per cent, a value which is equal to zero within the experimental error and corresponds to a pectic acid of 100 per cent polygalacturonide.

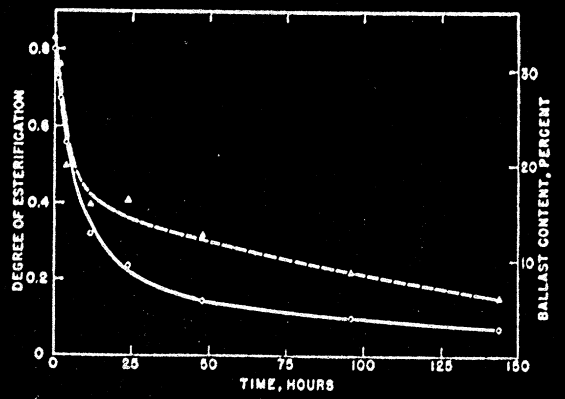

FIG. 3. Acid deësterification of pectin sample H84. Temperature, 50°C.; pectin concentration, 1.3 per cent. ○ = degree of esterification; △ = ballast content.

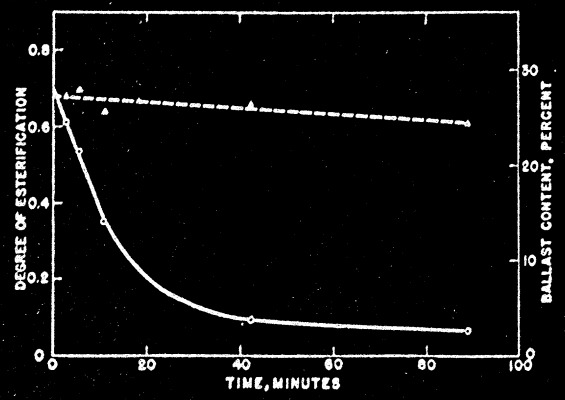

FIG. 4. Enzyme deësterification of pectin sample H87. Temperature, 50°C.; pectin concentration, 1.2 per cent. ○ = degree of esterification; △ = ballast content.

In figure 5 are plotted the logarithms of degree of esterification and of ballast content *versus* time for the same acid-deësterified samples illustrated in figure 3. The demethylation curve is linear for degree of esterification greater than about 0.5 ($\log \lambda = 0$ to -0.3), showing that the rate of demethylation is first order with respect to concentration of methyl ester. This means that the over-all reaction is pseudo first order, with concentrations of water and catalyst substantially constant. From the linear portions of this curve and similar curves for other

series of samples, pseudo first-order rate constants were calculated and are listed in table 1.

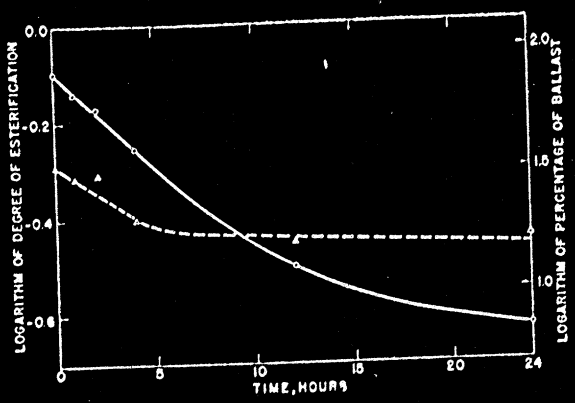

FIG. 5. Acid decësterification of pectin sample H84. Temperature, 50°C.; pectin concentration, 1.3 per cent. ○ = logarithm of degree of esterification; △ = logarithm of ballast content.

TABLE 1

Rate constants for demethylation of pectin as a function of temperature

Acid-catalyzed, pseudo first order; pectin concentration, 1.3 per cent; HCl, 0.87 *N*

TEMPERATURE	SERIES	$k \times 10^4$
°C.		$sec.^{-1}$
30	H86	0.316 ± 0.044
40	H91	1.039 ± 0.15
50	H84	2.543 ± 0.36
60	H85	4.201 ± 0.59

Enzyme-catalyzed, pseudo zero order; pectin concentration, 1.2 per cent; pH, 6.00

TEMPERATURE	SERIES	$k \times 10^4$
°C.		$sec.^{-1}$
30	H89	2.85 ± 0.10
40	H88	3.88 ± 0.04
50	H87	5.18 ± 0.23

Enzyme-catalyzed, pseudo first order; pectin concentration, 1.2 per cent; pH, 6.00

TEMPERATURE	SERIES	$k \times 10^4$
°C.		$sec.^{-1}$
30	H89	5.75 ± 0.23
40	H88	8.55 ± 0.11
50	H87	12.15 ± 0.56

The logarithms of these rate constants are plotted against the reciprocal of the absolute temperature in figure 6, and the activation energy for this series

was calculated to be 17,400 ± 1300 cal. The slight curvature of the points is within the experimental error.

In figure 7 are plotted the logarithms of degree of esterification and ballast content *versus* time for the same enzyme-deësterified samples illustrated in figure 4. Both the degree of esterification and its logarithm appear to be linear functions of time for λ greater than about 0.3 (log λ = 0 to −0.5). Thus the probable error of our points is such that the data fit both the zero and the first orders

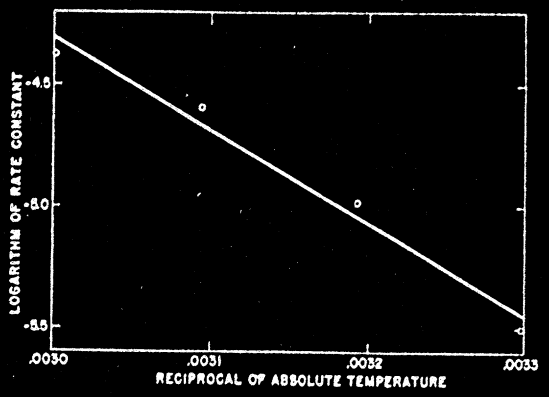

FIG. 6. Rate of acid demethylation of pectin as a function of temperature

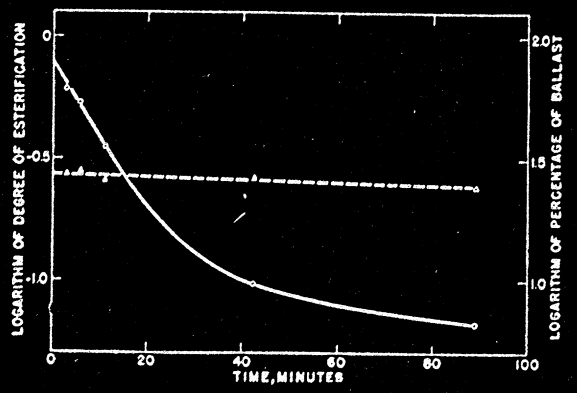

FIG. 7. Enzyme deësterification of pectin sample H87. Temperature, 50°C.; pectin concentration, 1.2 per cent. ○ = logarithm of degree of esterification; △ = logarithm of ballast content.

equally well. From the linear portions of these curves and of similar curves for other enzyme deësterifications, rate constants were calculated according to both orders and are listed in table 1.

Logarithms of these two sets of rate constants are plotted against the reciprocal of the absolute temperature in figure 8. Activation energies calculated from these data are 5790 ± 520 cal., if a pseudo zero order is assumed, and 7310 ± 590 cal. if a pseudo first order is assumed. In both cases the activation energy is constant over an interval of 20°.

The loss in ballast during enzyme demethylation is so slight that an analysis was not made of the enzyme ballast data; however, acid-catalyzed deësterification removes ballast fast enough to warrant quantitative treatment. From figure 5 it can be seen that the logarithmic curve for ballast removal is substantially linear over approximately the same range as the demethylation curve. The poorer fit of the ballast points is due to the method of calculating ballast content by difference and to the uncontrollable variations in the amount of loose ballast

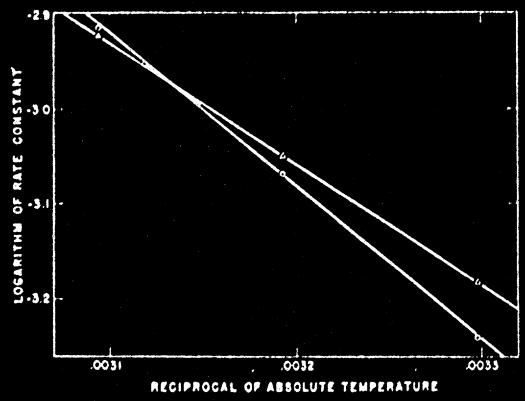

FIG. 8. Rate of enzyme demethylation of pectin as a function of temperature. ○ = logarithm of first-order rate constant; △ = logarithm of zero-order rate constant + 0.3622.

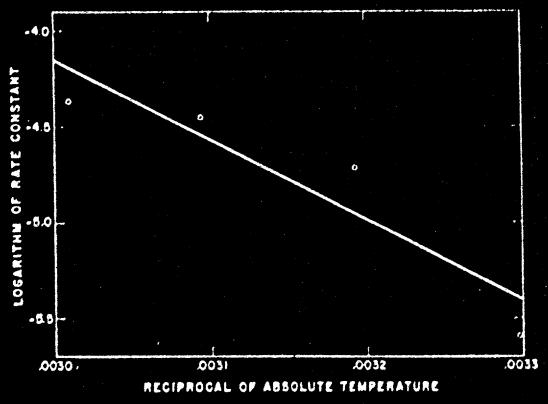

FIG. 9. Rate of ballast removal during acid deësterification, as a function of temperature

washed out in the purification process. The initial linearity of the logarithmic curve shows that ballast was removed as a chemical reaction obeying a first-order law. From the linear portion of this curve and from similar ballast curves, for the same series of samples and temperatures used in the demethylation experiments, rate constants were calculated and are shown in figure 9. From these values, the activation energy was calculated according to the method of least squares and was found to be 18,500 ± 4000 cal. Ballast not chemically attached to the galacturonide chain does not affect this calculation because of the standardized washing procedure used.

Table 2 lists pseudo first-order rate constants for acid demethylation of pectin at a fixed temperature for varying concentrations of pectin and of hydrochloric acid. The data for H103, H106E, and H106F show that the velocity constant is independent of the concentration of pectin, since each of the three rate constants differs from the weighted average by less than the experimental error. This is further evidence that the reaction is first order with respect to methyl ester concentration.

The data for varying acid concentrations are shown in figure 10. These rate constants fall on a straight line that passes through the origin, which shows that

TABLE 2

Pseudo first-order rate constants for acid demethylation of pectin as a function of pectin concentration and acid normality

(Temperature, 40°C.)

SERIES	CONCENTRATION OF PECTIN	CONCENTRATION OF ACID	$k \times 10^6$
	per cent	*N*	*sec.*$^{-1}$
H106D	2.5	0.1	0.963 ± 0.084
H106C	2.5	0.2	1.655 ± 0.156
H106B	2.5	0.3	2.67 ± 0.33
H103	2.5	0.6	6.35 ± 1.12
H106E	1.5	0.6	5.62 ± 0.34
H106F	0.5	0.6	6.25 ± 0.50
H106A	2.5	1.0	9.86 ± 0.34
Weighted average of H103, H106E, H106F		0.6	5.85 ± 0.28

the reaction is also first order with respect to acid concentration. This line was found, by least squares calculation, to follow the equation:

$$k = (9.62 \pm 0.24) \times 10^{-6}\,(H^+) \text{ at } 40°C. \qquad (4)$$

Using this value of k and 17,400 cal. for the activation energy, we have derived an empirical equation giving the rate of acid demethylation of pectin as a function of temperature and acid concentration:

$$-\frac{d(COOCH_3)}{dt} = (COOCH_3)(H^+) \times 1.35 \times 10^7 \exp\left(-\frac{17{,}400}{RT}\right) \qquad (5)$$

This should be useful in industrial practice, although it should be mentioned that, at high acid concentrations, temperatures above 70°C. may produce serious degradation of the pectin chain.

DISCUSSION

Acid deësterification

Our value for the activation energy for acid demethylation of pectin, 17,400 ± 1300 cal., is in good agreement with values appearing in the literature for

esters of low molecular weight, such as 17,100 cal. for methyl acetate (10) and 16,100 to 16,500 cal. for ethyl esters of normal fatty acids from acetate to caprylate (6, 34).

We found that at all temperatures the plots of log λ *versus* time began to deviate from linearity at a degree of esterification of approximately 0.5. An explanation

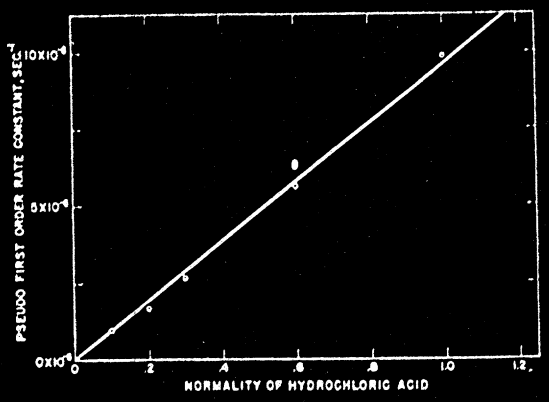

FIG. 10. Rate of acid demethylation of pectin as a function of acid concentration. Temperature, 40°C.

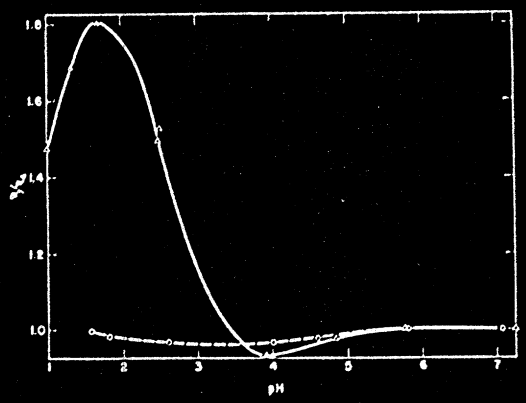

FIG. 11. Viscosity of partially deësterified pectins relative to viscosity at pH 6.0. Concentration, 0.6 per cent in aqueous solution; temperature, 30°C. ○ = sample H84C: degree of esterification, 0.577; ash content, 0.16 per cent. △ = sample H84D: degree o esterification, 0.317; ash content, 0.18 per cent.

for this phenomenon can be obtained from figure 11. In this figure, the viscosity of sample H84C (the last point on the linear portion of figure 5) is compared with the viscosity of sample H84D (the first point of figure 5 that deviated from linearity). The striking difference in viscosity behavior as a function of pH is typical of the difference between a pectin which will not gel in acid solution in the absence of sugar and a pectin which will. The same behavior is reflected in the formation of calcium pectinate jellies (14), where a rather sharp dividing line

occurs at $\lambda = 0.5$ between pectins which will form low-sugar calcium pectinate gels and those which will not.

This difference in the gelling behavior of samples H84C and H84D suggests that incipient gel formation in the reaction mixture is the cause of the departure from linearity of the logarithmic demethylation curves.

The deësterification of a polymethyl ester such as pectin can take place in one of two ways: (*1*) All the ester groups are attacked at the same rate, independent of the condition of esterification of nearby groups; or (*2*) the removal of a given methyl group is influenced by the condition of esterification of adjacent parts of the chain. These two types can usually be distinguished by a simple rate analysis, as it can readily be shown that Case 1 follows a pseudo first-order law, whereas Case 2 should have a more complex behavior.

The n^{th} stage in the hydrolysis of a pectin molecule containing s main-chain residues can be symbolized as

$$A_{n-1}M_{s-n+1} \xrightarrow{k_n} A_nM_{s-n} \tag{6}$$

where A refers to acid groups and M to methyl ester groups. The rate of loss of methyl groups for this stage of hydrolysis is

$$-\frac{d(COOCH_3)}{dt} = k_n(A_{n-1}M_{s-n+1}) \tag{7}$$

If Case 1 applies, such that each of the $(s - n + 1)$ methyl ester groups hydrolyzes independently and at the same rate v, then

$$k_n = (s - n + 1)v \tag{8}$$

and it follows that the observed over-all rate of hydrolysis, due to all stages combined, will be

$$-\frac{d(COOCH_3)}{dt} = \sum_{n=1}^{s} k_n(A_{n-1}M_{s-n+1}) = v\sum_{n=1}^{s}(s - n + 1)(A_{n-1}M_{s-n+1}) \tag{9}$$

But the total concentration of methyl ester groups in the system is

$$(COOCH_3) = \sum_{n=1}^{s}(s - n + 1)(A_{n-1}M_{s-n+1}) \tag{10}$$

Therefore

$$-\frac{d(COOCH_3)}{dt} = v(COOCH_3) \tag{11}$$

which is identical with the equation for a first-order reaction. A similar result can be obtained from the equations of Ingold (16) (assuming all v's equal) and has also been derived for the special case of dicarboxylic esters by Wegscheider (36).

We have found that acid deësterification of pectin obeys a pseudo first-order law and fits the Arrhenius equation well, which suggests that the hydrolysis of a given methyl ester group is independent of the condition of the rest of the mole-

cule (Case 1). This is in contrast to the acidic dissociation of pectinic acid, which was found (35) not to be independent of the degree of dissociation of the rest of the chain. In this respect the macromolecule, pectin, shows the same behavior as low-molecular-weight esters and acids. Many esters of dibasic acids are hydrolyzed according to the assumptions of Case 1, since k_1/k_2 is experimentally found (23) to be equal to 2, as required by equation 8. This is in contrast to the wide difference in order of magnitude usually observed between the first and second acid dissociation constants of dibasic acids, instead of the simple 4 to 1 ratio expected from independent ionization (see equation 5 of reference 35). For example, Meyer (23) found $k_1/k_2 = 1.98$ (theoretical 2.00) for the hydrolysis of dimethyl succinate, whereas Gane and Ingold (8) found $K_1/K_2 = 16.4$ (theoretical 4.0) for the dissociation of succinic acid.

A possible explanation for this difference in behavior can be found in the mechanism proposed by Datta, Day, and Ingold (5) for the hydrolysis of esters. The rate-determining step of their mechanism involves no net change in the number of electrical charges, whereas acidic dissociation and recombination produce and destroy charges. It should therefore not be necessary to do electrostatic work on the surrounding ionic atmosphere in ester hydrolysis, and the influence of dipoles in nearby molecules or in adjacent parts of the same molecule should have less effect on hydrolytic reactions than on electrolytic dissociations. A similar point of view has been put forward by Brönsted (2) and checked experimentally for ester hydrolysis by Dawson and Lowson (7), who found that the rate of hydrolysis of ethyl acetate is proportional to the concentration of hydrochloric acid rather than to its thermodyanic activity. For pectin, we also find that the rate of deësterification is proportional to hydrochloric acid concentration, as shown in figure 10, although our experimental error is too great to decide definitely between concentration and activity. Therefore, it is apparent from the above discussion that pectin hydrolysis follows Case 1, whereas its acidic dissociation follows Case 2.

Enzyme deësterification

The values 5790 ± 520 cal. and 7310 ± 590 cal. for the activation energy of demethylation of apple pectin by tomato pectase may be compared with 6000 cal. found by Owens, McCready, and Maclay (29) for demethylation of citrus pectin by citrus pectase *in situ*. Both are also comparable to activation energies found for several other esterases acting on diverse substrates, such as 7600 and 8500 cal. for pancreatic lipase (33, 32) and 5700 cal. for grasshopper esterase (3).

Under the conditions used in this set of experiments, enzymic deësterification fits either the pseudo zero-order law or the pseudo first-order law equally well. Other experiments by Hills and Mottern (12) suggest that tomato pectase follows the zero-order law better than the first-order law. On the other hand, Kertesz (19) found that tobacco pectase obeyed the first-order law up to 60 per cent of reaction. This point is not definitely settled and will be considered in more detail in a later paper (12).

Preliminary calculations applying the Michaelis–Menten theory (24) to other

unpublished data on tomato pectase indicate that the substrate concentrations used in experiments H87, H88, H89 fall in the intermediate range where it cannot be predicted from the theory which of the two orders of reaction should apply. There is also some question whether the Michaelis-Menten equations in their present form should be applied to a high polymeric system such as pectin, where several groups in the same molecule may be reacting simultaneously. For these reasons, we are including calculations according to both orders.

Since the data follow regular reaction-rate laws up to 50 per cent of reaction and fit the Arrhenius equation well, enzymic deësterification appears to follow Case 1 along with acid deësterification. However, electrophoretic data and gel strengths (to be reported in a later paper) indicate that, unlike acid deësterification, the methyl ester groups are not attacked at random. Likewise, Kertesz (18) found that molecular size may be an important factor in enzyme deësterification of pectin, and Lineweaver, Jansen, Owens, *et al.* (20) have suggested that the action of pectase is more selective than alkaline hydrolysis. This suggests that, although the *selection* of a methyl group for enzymic removal depends on the condition of the neighboring parts of the chain, the *rate* of enzymic removal of a methyl group is not dependent on the nearby parts of the molecule. Differences in mechanism between acid and enzyme deësterification are not surprising in the light of the highly specialized character of enzyme catalysis.

Ballast removal

Hirst and Jones (15) concluded that the ballast material in pectin is not joined to the main polygalacturonide chain by covalent chemical bonds but is merely loosely associated by secondary valences, in general agreement with Schneider and Bock (31). On the other hand, Norris and Resch (27) were of the opinion that a part of the non-galacturonide material may be attached to the main chain by primary bonds, for they have stated, ". . . it is probable that the usual sample of pectin consists of a central nucleus of galacturonic acid units to which arabinose and galactose units may be chemically attached. . . . In addition to this there must be varying amounts of araban and galactan or arabogalactan which are held to the main nucleus by physical forces" (27).

Re-analysis by us of the data of Hirst and Jones (15) does not confirm their conclusion that only loose association forces are involved. Although they found that a portion of the ballast was easily washed out in 70 per cent alcohol, their data also showed that alkaline treatment sufficiently harsh to remove all the methyl ester still left 15–20 per cent of the original galactan, as shown by their final equivalent weight of 185 and uronic anhydride content (by carbon dioxide liberation) of 96.7 per cent. Similarly, Norris and Resch (27) found that treatment of hops pectin for 193 hr. with 4 per cent sodium hydroxide (which would remove most of the methyl ester) still left araban and galactan contents of 3.8 and 7.7 per cent, respectively.

Experimentally, we find that, after removal of loose ballast by the washing procedure described in the experimental section, a substantial quantity of ballast remains that requires an activation energy of 18,500 cal. for its removal, which

lends evidence to the theory that covalent bonds are involved. Polysaccharide ballast could be chemically attached to a polyuronide chain by ether, ester, anhydride, or hemiacetal linkages, assuming the necessary end groups on the ballast chain. The anhydride and hemiacetal linkages are ruled out immediately by their instability in the aqueous environment in which pectin occurs naturally. The ether linkage is also ruled out because of its high bond strength of at least 29,000 to 35,000 cal. (11, 25). The ester linkage is a possibility because its bond strength is in the neighborhood of the 18,500 value we obtain.

Our method of calculating degree of esterification and ballast content is based on the assumption that the sum of the methyl esters and free carboxyls is a true measure of the number of galacturonide units present. If some of the galacturonide units have their carboxyl groups tied up by ester linkages to ballast, the sum of methyl ester groups plus free carboxyl groups will not be equal to the number of galacturonide units. However, the molecular weights of araban and galactan are so high (6000 and higher (9, 26)) that the error introduced into the carboxyl content will be too small to detect, except by elaborate end-group analysis.

Another possible way to account for the nature of the ballast-removal curves we obtain is that all the ballast is merely physically mixed with the polygalacturonide but that a portion is of such high molecular weight that it is only difficultly soluble in the 80 per cent alcohol used in the purification process. In this case the increasing amount of ballast washed out by the alcohol after deësterification must be accounted for by degradation of the ballast. This possibility is eliminated because the activation energy for ballast removal would then be found to be of the order of magnitude of 30,000 cal., as for degradation of other polysaccharide materials (30).

There have been suggestions that the araban and galactan are part of the main chain, occurring between polygalacturonide sections. Two facts rule this out: (*1*) The molecular weight of the ballast is so high that its removal from the midst of the main chain would result in a very rapid falling off of viscosity, which is not observed, and (*2*) by the continuation of acid hydrolysis for sufficiently long periods, the ballast content can be reduced to substantially zero, as shown in figure 3. The molecular weights of samples thus treated are still at least as high as 30,000, as indicated by viscosity, gel formation, and film formation.

Hence it is our conclusion that a substantial fraction of the ballast is probably attached to the polygalacturonide chain by ester linkages. The remainder may be merely physically mixed with the polygalacturonide or attached by secondary valences such as hydrogen bonds. One is strongly tempted to say that the loosely bound material is araban and that the chemically bound material is galactan, in the light of findings of Hirst and Jones (15) that mild hydrolytic means removed practically all the araban but hardly affected the galactan. However, two facts should be noted: (*1*) Their washing procedure with 70 per cent alcohol removed both araban and galactan at approximately equal rates but did not completely remove either; (*2*) their hydrolytic treatment that

removed araban almost completely was conducted under such conditions that the araban was degraded to arabinose. Since the furanose structure of araban makes it very susceptible to hydrolysis (15), there still remains the possibility that the araban was joined to the polygalacturonide by an ester linkage and that the arabinose was produced by degradation of the araban while still attached, leaving the last arabinose residue esterified to the polygalacturonide. Because of the high molecular weight of araban, the amount of arabinose thus left with the galacturonide would be too small to be detected, except by the methods of end-group analysis.

SUMMARY

The kinetics of acid- and enzyme-catalyzed deësterification of apple pectin has been studied.

Rate constants were measured for acid demethylation at hydrochloric acid concentrations from 0.1 to 1.0 *N*, at temperatures from 30° to 60°C., and at pectin concentrations from 0.5 to 2.5 per cent. Under these conditions, the reaction was found to be of pseudo first order, with an activation energy of 17,400 ± 1300 cal. The rate of acid demethylation, over the intervals of temperature and concentration used, can be expressed as an empirical equation:

$$-\frac{d(COOCH_3)}{dt} = (COOCH_3)(H^+) \times 1.35 \times 10^7 \exp\left(-\frac{17{,}400}{RT}\right)$$

Rate constants were measured for demethylation catalyzed by tomato pectase at temperatures from 30° to 50°C. and at a pectin concentration of 1.2 per cent. The data fit the pseudo zero-order and the pseudo first-order laws equally well. Assuming zero and first orders, activation energies of 5790 ± 520 cal. and 7310 ± 590 cal., respectively, were calculated.

Analysis of the kinetics of removal of organic non-galacturonide material (ballast) during acid deësterification suggests that, in apple pectin, a substantial portion of the ballast is attached to the polygalacturonide chain by primary covalent bonds (probably ester linkages) having an activation energy of 18,500 ± 4000 cal. The remainder of the ballast is held by secondary valence forces or merely included as a physical mixture.

By extensive acid hydrolysis, ballast can be completely removed, leaving a stripped polygalacturonic acid chain that is still of high molecular weight and has an average residue weight approaching 176. In enzyme hydrolysis, very little ballast is removed.

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